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Mechanistic Basis of Calmodulin Mediated Estrogen Receptor Alpha Activation and Antiestrogen Resistance

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14. ABSTRACT

Estrogen receptor alpha (ERa) is the principle chemotherapeutic target for estrogen dependent breast cancers. Calmodulin (CaM) is an obligatory ERa activator. Moreover, antiestrogens (tamoxifen) bind tightly to CaM, and some therapeutic benefits of antiestrogens for breast cancers are hypothesized to derive from this interaction. The purpose and scope of the research is to define the structural requisites of ERa activation by CaM and the relationship between tamoxifen binding to CaM, CaM oxidation and antiestrogen resistance. We have localized and refined our understanding of the CaM binding sites on ERa. We demonstrated that the high affinity CaM binding region of ERa forms both helical and random coil structure when bound to CaM. We demonstrated that tamoxifen, hydroxytamoxifen and raloxifene binding to CaM are eliminated when the methionine residues of CaM are oxidized. We determined that oxidation of the methionine residues in CaM does not eliminate CaM binding to ERa. The results suggest a mechanism whereby antiestrogen resistance is exacerbated by oxidative stress.

15. SUBJECT TERMS
estrogen receptor alpha, calmodulin, activation, oxidative stress, antiestrogen resistance, tamoxifen, structure, NMR

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***a cv is attached following "Supporting Data", on pages 20-42**

INTRODUCTION:

Estrogens and the alpha isoform of the estrogen receptor (ERa) are central to estrogen-dependent breast cell carcinoma induction and proliferation. The principal target for systemic endocrine/antiestrogen therapy is ERa, underscoring its biological relevance and medical importance. Despite the apparent wealth of functional and structural information on ERa, the molecular mechanism of ERa activation is sorely incomplete, as only recently has it been established that calcium-dependent activation by calmodulin (CaM) is essential for estrogen-dependent ERa activity, and that the true “active” species is the CaM-ERa complex. This unexpected result has left a glaring gap in our fundamental understanding of ERa activation. CaM also binds tightly to antiestrogens, including the most widely used chemotherapeutic agent for estrogen-dependent breast cancers, tamoxifen (TAM). The therapeutic effects of antiestrogens, like those of other CaM antagonists, are indicated to be due, in part, to the direct interaction with CaM. Furthermore, conditions of high oxidative stress and high levels of reactive oxygen species in breast cancer tissues, which are closely linked to antiestrogen resistance, result in oxidation of important methionine residues in CaM, resulting in accumulation of oxidized CaM species and altered function. Finally, CaM is directly implicated in the observed increased tamoxifen resistance associated with unusually high protein kinase A (PKA) levels, as PKA phosphorylates a serine (Ser) residue in the CaM binding domain of ERa, resulting in structural changes in ERa. Because CaM is essential for ERa activation, because of the mounting evidence for the direct involvement of CaM in antiestrogen therapy and antiestrogen resistance, structural and mechanistic details of CaM interactions with ERa and antiestrogens, and the role of posttranslational modifications (CaM oxidation, ERa phosphorylation) on CaM regulation of ERa must be a high priority. Thus, the scope of our research is to define the molecular mechanism, including the structural details, by which CaM activates estradiol-dependent ERa transcription, to demonstrate and define the role of oxidative stress in mediating CaM-ERa and CaM-antiestrogen interactions, and to establish and characterize the role of CaM in PKA-induced antiestrogen resistance

BODY:

Task 1: Define the molecular mechanism, including the structural details, by which CaM activates estradiol-dependent ERa transcription (Months 1-36).

The overall goal here is to describe, from a structural perspective, how CaM binding to ERa activates the receptor. Our principle structural tool is NMR spectroscopy.

<u>Subtask a).</u> *Produce ERa and CaM (isotopically labeled and unlabeled) for NMR studies (Months 1-8).* We demonstrated previously ("Preliminary Results" of our proposal) the ability to produce a construct of ERa that includes the putative CaM binding region and the ligand binding domain of ERa (residues 286-552, see **Figure 1** for estrogen receptor structural organization), and we demonstrated that this construct binds CaM. We have also produced isotopically labeled samples of this ERa construct for preliminary NMR studies.

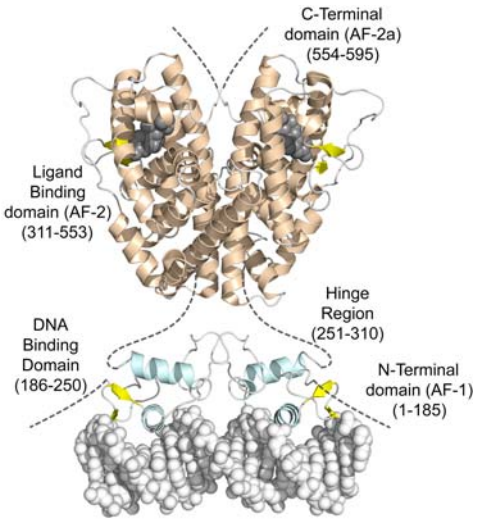

Figure 1: Structural organization of the estrogen receptor dimer. The X-ray crystal structure of the LBD (tan) of ERα with estrogen (gray, space-filling) bound is from Gangloff et al. (*1*) (PDB identifier 1QKU). The X-ray crystal structure of the DBD (blue) of ERα bound to DNA is from Schwabe et al. (*2*) (PDB identifier 1HCQ). No high resolution structural information is available for the AF-1, AF-2a and hinge domains. We have determined the putative CaM binding region is at the C-terminal end of the hinge region (residues 287-311), which includes K302, K303 and S305, all ofwhich can be posttranslationally modified (lysines acetylated, serine phosphorylated).

As stated in the previous report, we have not been able to record acceptable NMR spectra of isotopically labeled ERa (with either E2 or TAM bound) or the ERa-CaM complex (this concerns "*Subtask b*", below). Our goal was to produce ERa purified by affinity chromatography on immobilized CaM resin that had not been carboxymethylated with iodoacetic acid. Carboxymethylation of cysteine residues in ERa for structural studies has been adopted nearly universally, but we hoped to avoid this in order to more closely mimic the native state. We currently are pursuing an alternate production and purification strategy that involves affinity purification on estradiol sepharose (**Figure 2**). We are also introducing a control construct (residues 302-552) that does

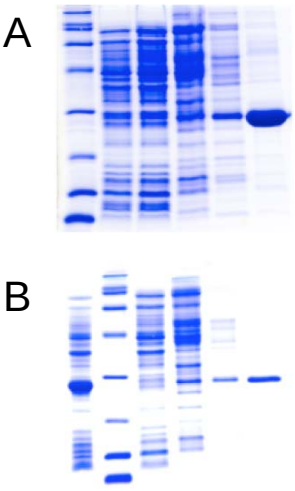

Figure 2. A). Analysis of His_6-ERa(302-552) Purification. Histidine-tagged ERa ligand binding domain, encompassing residues 302-552, was selectively purified from crude bacterial lysate by affinity chromatography on Estradiol Sepharose 6B. Purification of His_6-ERa(302-552) was performed under denaturing conditions. Fractions were collected at each purification step and subjected to SDS-PAGE (15%). Lane 1: molecular weight marker. Lane 2: E_2-Sepharose column flow-through. Lanes 3-5: wash fractions 1 through 3, respectively. Wash buffer 1 contained 1 M urea, and wash buffers 2 and 3 contained 3 M and 5 M urea. Lane 6: elution of His_6-ERa(302-552) with buffer containing 5 M urea and 50 mM E_2. **B)**. Analysis of ERa(286-552) Purification. ERa residues 286-552 was purified under denaturing conditions by affinity chromatography on Estradiol Sepharose 6B. All buffers contained differing concentrations of urea. Fractions were collected at each purification step and subjected to SDS-PAGE (15%). Lane 1: whole cell bacterial lysate. Lane 2: molecular weight marker. Lane 3: E_2-Sepharose wash fraction 1. Lane 4: wash fraction 2. Lane 5: wash fraction 3. Lane 6: pure ERa(286-552). Wash buffers 1-3 contained 1 M, 3 M, and 5 M urea, respectively. The elution buffer contained 5 M urea with 50 mM E_2.

not include the CaM binding region in order that we might more easily evaluate any deleterious effects on production/purification/stability of the presence of the Cam binding region.

Subtask b). *Perform NMR experiments on the complex between CaM and ERa with E2 (estrogen) bound and the CaM-ERa complex with TAM bound (Months 8-18).* As discussed above under "*Subtask a*", we have not yet been able to record acceptable spectra of our ERa construct that includes the CaM binding domain and the ligand binding domain (residues 286-552), and we currently are pursuing an alternate ERa production and purification approach.

As described in the previous report, we have also initiated a complementary, but more limited, approach to understanding some of the structural aspects of the interaction between ERa and CaM. In this approach we are defining the structural details of the complex of CaM with the CaM binding region only of ERa. In general, these types of studies of CaM interactions with binding domains from protein targets have been very successful (*3, 4*). Thus, this approach will enable some of the important structural aspects of this interaction to be resolved. This component is well underway, as indicate in the previous report, and should be completed this year.

As described in the previous report, we wanted to confirm our localization of the CaM-binding region of ERa. Our strategy was to examine CaM binding to different segments of the hinge region of ERa (and N-terminal end of the ligand binding domain). In order to do this, we produced two constructs to study their interactions with CaM. The first was a protein construct consisting of the hinge region of ERa and the N-terminal end of the ligand binding domain (residues 241-320) fused to an affinity tag (His_6-tag) and thioredoxin for solubility (**Figure 3**). We call this protein Trx-$ERA_{241-320}$. We also produced a similar protein construct, but with only residues 241-273 of ERa, as a "control" (**Figure 3**). We call this protein Trx-$ERA_{241-273}$. Because CaM binds tightly to the N-terminal extended ligand binding domain of ERa (residues 286-552, see above), we hypothesized that Trx-$ERA_{241-320}$ would bind tightly to CaM, but that Trx-$ERA_{241-273}$ would not.

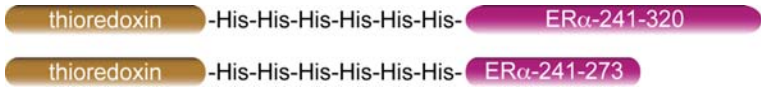

Figure 3. Schematic representations of the proteins Trx-$ERA_{241-320}$ (top) and Trx-$ERA_{241-273}$ (bottom), used in studies to localize the CaM binding region of ERa.

In the previous report we detailed our results with these constructs, and determined that the main CaM binding region of ERa is residues 287-311, but there was some evidence of an additional low affinity site in the region from 241-273. In order to address the existence of this putative weak site, we produced two additional control Trx constructs, one called Trx-RNDM consisting of Trx-$ERA_{241-273}$ where the amino acid sequence of the region 241-273 was randomized. The other control construct was Trx alone (including the His_6 region). These were produced and purified (**Figure 4**) in a manner very similar to the

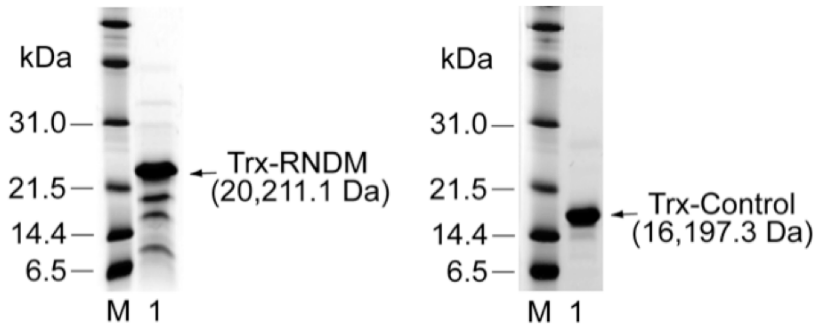

Figure 4: (**Left**). SDS-PAGE assessment of the production/purification of Trx-RNDM (Trx-$ERA_{241-273}$, where the amino acid sequence 241-273 was randomized). The protein was produced as described previously for our other Trx constructs and purified by IMAC chromatography. The indicated mass is the exact mass. (**Right**). SDS-PAGE assessment of the production/purification of Trx-Control (Trx only). The protein was produced as described previously for our other Trx constructs and purified by IMAC chromatography. The indicated mass is the exact mass.

initial constructs (see previous year report). The results of binding to CaM are shown in **Figure 5** and **Figure 6**.

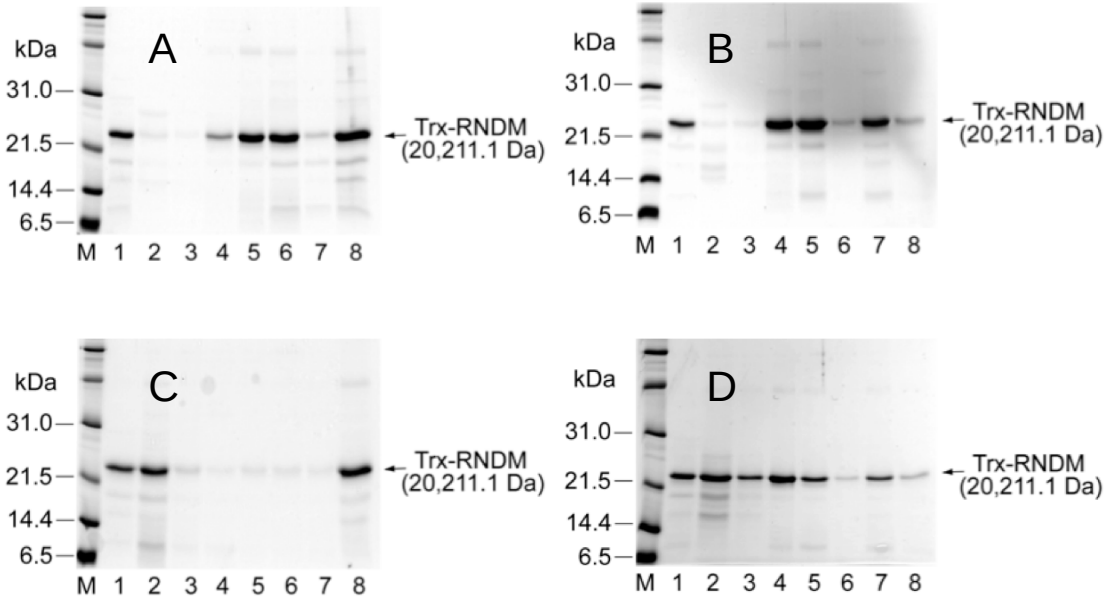

Figure 5: Trx-RNDM (Trx-ERA$_{241-273}$, where the amino acid sequence 241-273 was randomized) binds weakly, nonspecifically to CaM. SDS-PAGE was used to analyze eluents from immobilized CaM resin (or control resin without immobilized CaM) for affinity of Trx-RNDM for the resins either in the presence or absence of Ca^{2+}. (A) In the presence of Ca^{2+}, Trx-RNDM binds apparently to immobilized CaM (CaM-Sepharose 4B), is mostly released by high ionic strength, with some remaining protein eluted by addition of a Ca^{2+} chelator (EDTA). (B) When no Ca^{2+} is present, there is still some apparent affinity of Trx-RNDM for immobilized CaM, but most is eluted from the immobilized CaM by high ionic strength. (C)/(D) These are controls for 'A' and 'B', where resin (Sepharose 4B) alone, without immobilized CaM, is used. The results in C particularly indicate non-specific interaction of this construct with the resin, and not with the calmodulin.For 'A' and 'C', the equilibration buffer for the affinity resin and the buffer solution for the CaM is 20 mM Tris-HCl, pH 7.5, with 2 mM Ca^{2+}, and the elution buffer is 20 mM Tris-HCl, pH 7.5, with 2 mM EGTA and 1 mM $NaCl_2$. For 'B' and 'D', the equilibation buffer (and the buffer solution for the CaM is 20 mM Tris-HCl, pH 7.5, with 1 mM EDTA, and the "elution" buffer is 20 mM Tris-HCl, pH 7.5 with 1 M NaCl and 10 mM $CaCl_2$. M=molecular weight markers. Lane 1, purified Trx-ERA$_{241-320}$ protein in equilibration buffer. Lane 2, load flow-through. Lane 3, equilibration buffer. Lanes 4-7, equilibration buffer with 100, 200, 500, or 1000 mM NaCl, respectively. Lane 8 is elution buffer.

The results in **Figure 5** and **Figure 6** indicate a non-sequence specific interaction between residues 241-273 of ERa and CaM, and a very low affinity of Trx with CaM. Thus, the putative interaction that we proposed between residues 241-273 and CaM is simply a non-specific interaction. The affinity between ERa and CaM is, therefore, due to the interaction of CaM and the region of ERa comprising residues 287-311.

As we described in the previous report, based on the well-known propensity of CaM to bind to basic, amphiphilic regions, and based on our results (above) showing that CaM binds with high affinity to an ERa ligand binding domain located between residues 274 and 320 (see above), and we hypothesized the existence of a high-affinity CaM binding domain comprised of residues 287-311 of ERa (**Figure 7**). We verified this, and also demonstrated that a shorter peptide (residues 295-311) did not bind as tightly to CaM, and therefore deduced that previous contentions by other researchers of the localization of this domain were incorrect (*5, 6*).

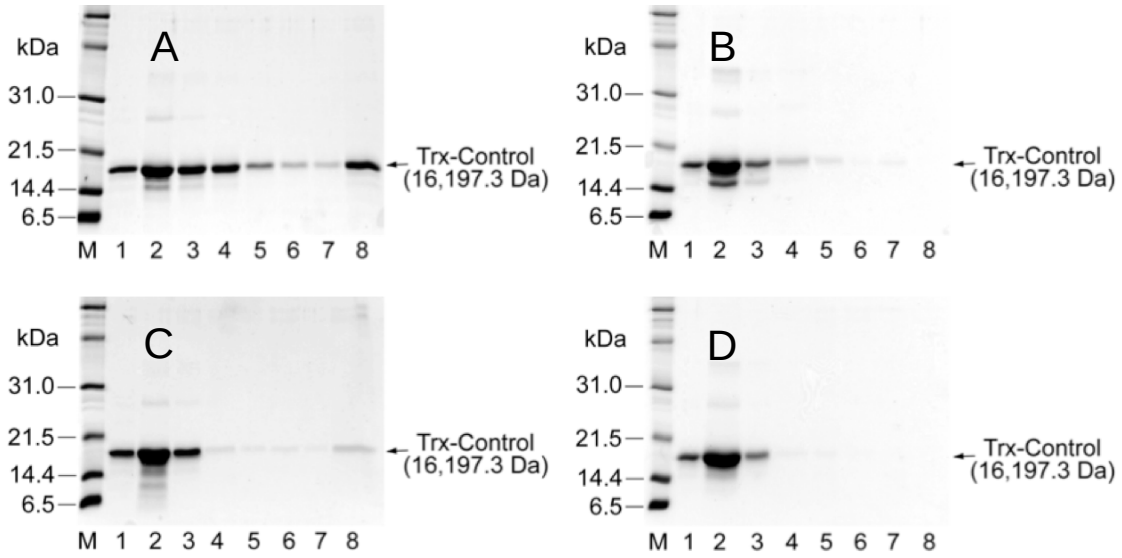

Figure 6: Trx does not bind specifically to CaM. SDS-PAGE was used to analyze eluents from immobilized CaM resin (or control resin without immobilized CaM) for affinity of Trx for the resins either in the presence or absence of Ca^{2+}. These experiments were performed in a manner identical to those in **Figure 5**. The panels here also correspond to those in **Figure 5**. There is a modest affinity of Trx for CaM in the presence of Ca^{2+} (A), but it is abrogated by high ionic strength

MLCK	**ARRKWQKTGHAVRAIGRLSS**
HERA$_{287-311}$	**287-RAANLWPSPLMIKRSKKNSLALSLT-311**
HERA$_{295-311}$	**PLMIKRSKKNSLALSLT**

Figure 7: Amino acid sequences of peptides used in these studies. Shown are sequences corresponding to residues 287-311 of the human estrogen receptor alpha (HERA$_{287-311}$), residues 295-311 (HERA$_{295-311}$), and the high affinity CaM binding sequence of myosin light chain kinase (MLCK), which is used as a control. Blue, positively charged. Orange, hydrophobic.

As detailed in the previous report, we are performing a variety of additional studies to characterize the interaction between CaM and HERA$_{287-311}$. For instance, in Figure 8 are shown results of circular dichroism that define structural attributes of the HERA$_{287-311}$ peptide when bound to CaM. The control smMLCK peptide adopts nearly complete helical structure when bound to Cam. However, HERA$_{287-311}$ adopts both helical and random coil structure when bound

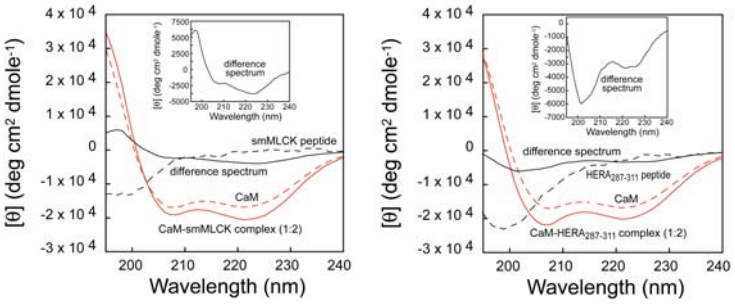

Figure 8. Circular dichroism spectra of CaM, CaM binding peptides, and complexes of CaM with these peptides. The control, CaM binding to the smMLCK peptide indicates considerable helical structure adopted by the peptide when bound (difference spectra). With HERA, the peptide adopts both helical and extended random coil structure when bound to CaM.

to CaM. This suggests perhaps that the canonical structure of CaM bound to tight binding amphiphilic regions is not represented by the CaM-HERA structure.

In the previous report, we detailed efforts to determine a high resolution structure of CaM bound to HERA$_{287-311}$. These studies are continuing. Towards this goal, we initiated production of a recombinant form of HERA$_{287-311}$ so that we could isotopically label it easily. Once labeled,

determining the structure of the bound peptide (bound to CaM) is much easier using NMR. We produced such a peptide using an intein construct, and purified it to homogeneity. However, it was determined by mass spectrometry that the peptide was a mixture of species 14 mass units different. Using MS/MS techniques, we determined that the peptide was modified at the single tryptophan residue, and is most likely methylated or some type of oxo-lactone. The ratio of the modified to correct species is almost exactly 1:1. We currently are deciding whether this approach will succeed (i.e. if we can produce the peptide using this expression system, or if we will have to try another).

<u>Subtask c).</u> *Calculate structures of the complexes using standard NMR computational methods and software (Months 12-36).* Hopefully, this subtask will be initiated this year. We anticipate calculating structures of CaM with bound $HERA_{287\text{-}311}$, and hope to be calculating structures of CaM bound to $HERA_{286\text{-}522}$.

Task 2: Demonstrate and define the role of oxidative stress in mediating CaM-ERa and CaM-antiestrogen interactions (Months 6-36).

The overall goal here is to determine if oxidative stress and oxidation of CaM resulting from oxidative stress can mediate the complex between CaM and ERa and antiestrogen resistance.

<u>Subtask a).</u> *Produce ERa and wildtype and mutant CaM proteins (isotopically labeled and unlabeled) for NMR, gel shift, and SPR studies (Months 6-18).* This is completed.

Subtask b). Perform NMR experiments on the complex between CaM and TAM (Months 6-24). Recent results have shown that TAM metabolites, rather than TAM itself, are the therapeutically

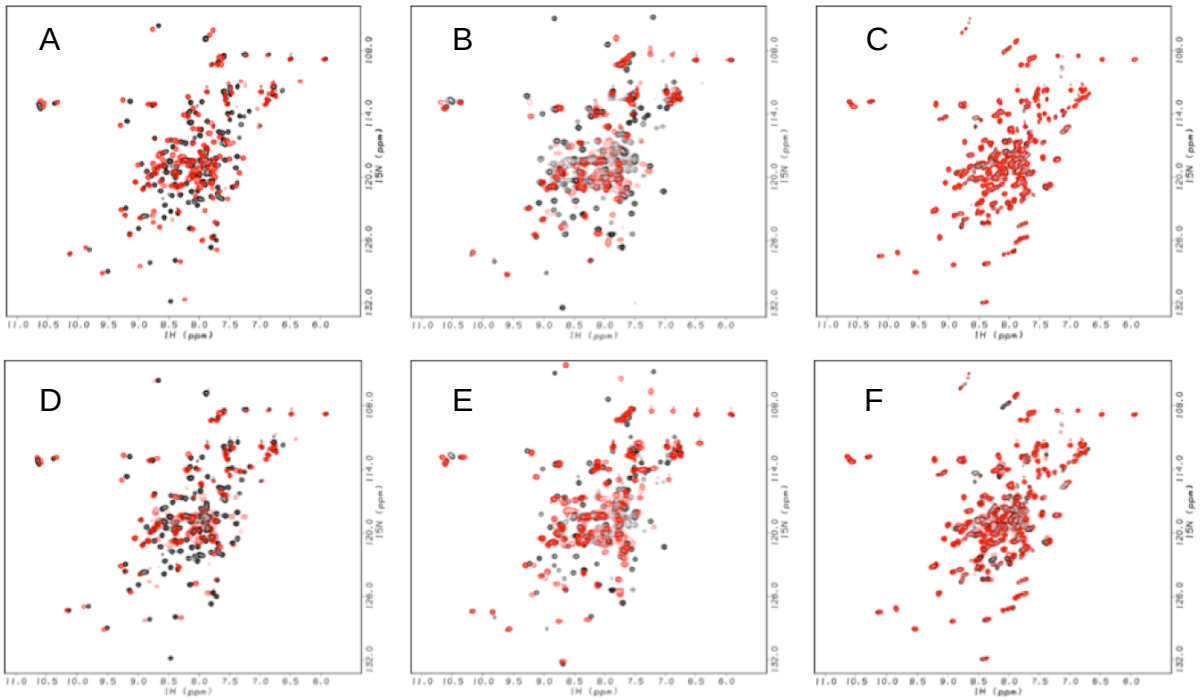

Fig. 9 ^{1}H, ^{15}N-HSQC spectra of ^{15}N, ^{13}C-labeled CaM species. A) Black, CaM. Red, CaM-OHTAM complex. B) Black, L9-CaM. Red, L9-CaM-OHTAM complex. C) Black, CaMox. Red, CaMox plus OHTAM. D) Black, CaM. Red, CaM-raloxifene complex. E) Black, L9-CaM. Red, L9-CaM-raloxifene complex. F) Black, CaMox. Red, CaMox plus raloxifene. The concentration of CaM or CaM species was ~0.5 mM, in 10 mM $CaCl_2$, 10 mM KCl, 5 mM d_4-imidazole, 0.02% sodium azide, 8% D_2O, pH 6.5.

active compounds responsible for the therapeutic benefits of TAM therapy (*7-9*). We have demonstrated that oxidative stress, if methionine oxidation in CaM results, can cause CaM to lose its ability to bind TAM. Our hypothesis is that CaM is no longer inhibited (CaM inhibition contributing to ERa deactivation), and can once again activate TAM (a mechanism for antiestrogen resistance brought about by oxidative stress). We now are continuing these studies of the effect of CaM oxidation on CaM binding to hydroxy tamoxifen (OHTAM), raloxifene (a different class of antiestrogens), and endoxifen (OHTAM and endoxifen are, putatively, the active metabolites of TAM). As shown in **Figure 9**, oxidation of CaM eliminates the interaction with OHTAM. This is the same result observed with TAM that we reported earlier. The control protein, CaM with all methionine residues substituted by leucine, binds OHTAM as well as wild type CaM. We also demonstrate in **Figure 9** that raloxifene binds to CaM (the first demonstration of this, to our knowledge), and that oxidation eliminates CaM binding to raloxifene also (there is, apparently, a very, very weak interaction of CaMox with raloxifene, most likely a non-specific interaction). These results are important and lend attitional credence to our hypothesis concerning CaM oxidation and antiestrogen resistance.

We also plan to perform these types of studies with endoxifen. There is only a single commercial source for endoxifen, and we placed an order for this compound long ago. We just recently received this compound, and will begin these studies shortly.

Finally, we originally had proposed to determine a structure of CaM with TAM bound. Based on the results shown in **Figure 9**, the complex of CaM with OHTAM is much more amenable to NMR than is the CaM-TAM structure. This appears to be due to the fact that OHTAM is slightly more soluble than TAM, and we can better saturate CaM with OHTAM in aqueous solution. Given that recent studies suggest endoxifen even more potent therapeutically, once we perform these experiments with endoxifen, we will choose to determine the high resolution structure of either the CaM-OHTAM or the CaM-endoxifen complex, depending on which gives the best spectra and is the best behaved in solution for NMR experiments.

Subtasks c-f). We will begin these in the next year.

Task 3: Test and describe the direct link between PKA induced antiestrogen resistance and CaM binding to ERa (Months 12-36).

The goal of this task is to test the hypothesis that posttranslational modifications in the CaM binding region of ERa mediate the interaction, and how this might contribute to antiestrogen resistance.

Subtask a). Complete

Subtask b). Complete

Subtasks c-e). In order to begin to address the overall aim of antiestrogen resistance and CaM oxidation, we need to determine if oxidized CaM (CaMox) still binds to (and activates) ERa. Towards this goal, initial experiments were performed using fluorescence spectroscopy to monitor the interaction between CaM, L9-CaM, and CaMox with the $HERA_{287-311}$ peptide. These results are shown in **Figure 10** and **Figure 11**.

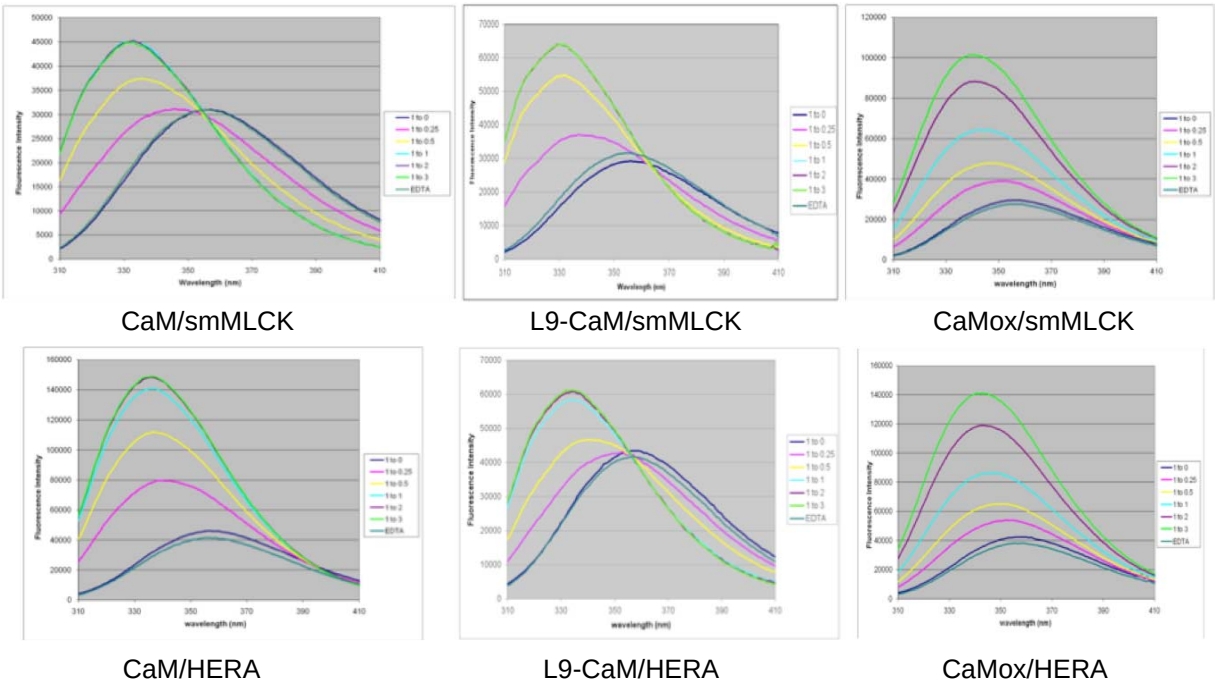

Fig. 10. Titration of HERA (the CaM binding domain of human estrogen receptor alpha, residues 287-311) and smMLCK (the CaM binding domain of the smooth muscle myosin light chain kinase) with CaM, L9-CaM (CaM with all methionine residues replaced with leucines) and CaMox (CaM with all methionine residues oxidized to the sulfoxides). The fluorescence spectra of the single tryptophan residues in HERA and smMLCK were acquired in the presence of saturating Ca^{2+} as the ratio of CaM/peptide was increased up to 3:1. At the conclusion of each titration, EDTA was added to demonstrate the Ca^{2+}dependence of binding. The peptide concentration was 10 mM and was held constant during the titrations. The excitation wavelength was 295 nm.

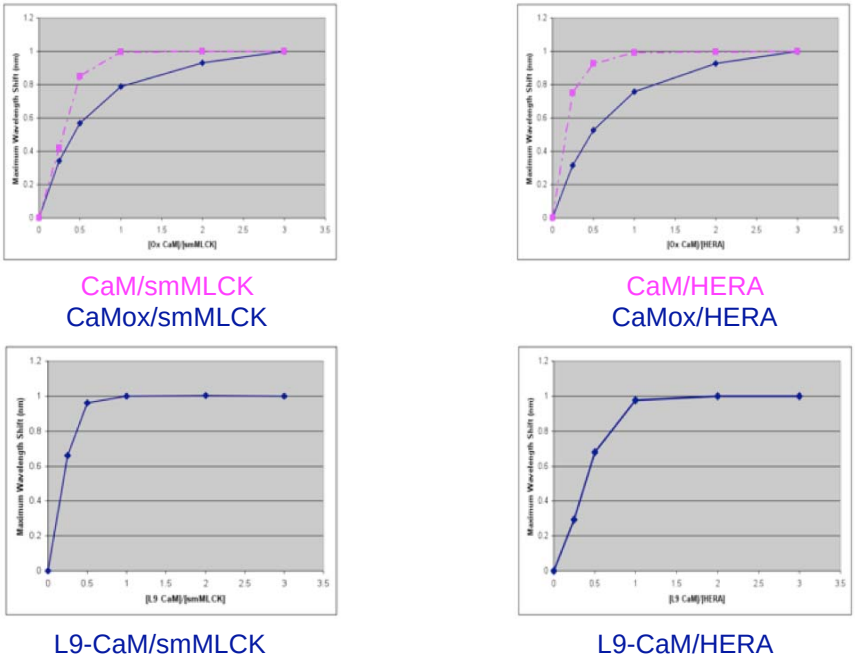

Fig. 11. Relative affinities of CaM, L9-CaM and CaMox for smMLCK and HERA. The maximum fluorescence emission wavelength shifts in the spectra in Figure Y were plotted as a function of the ratio of CaM species concentration to peptide concentration. The results indicate that oxidation of CaM reduces its affinity for smMLCK and HERA, but does not abrogate the interaction. L9-CaM also binds tightly to both smMLCK and HERA.

The results in **Figure 10** and **Figure 11** indicate that oxidation of the methionine residues in CaM decreases the affinity of CaM for $HERA_{287-311}$, but not to a limited degree. The results suggest that CaMox most likely can still activate ERa, thus, oxidative stress most likely cannot eliminate activation of ERa by CaM. This is a key result, and suggests that blocking ERa activation by CaM with TAM (or its metabolites) can be reversed by oxidative stress, and therefore could be a component of oxidative stress induced antiestrogen resistance.

Finally, we have initiated fluorescence and NMR experiments with modified (phosphorylated, acetylated) peptides. Results will be forthcoming.

KEY RESEARCH ACCOMPLISHMENTS (cumulative):

- *Residues 286-552 of ERa, which includes the entire ligand binding domain and the putative calmodulin binding domain, does indeed bind calmodulin*. One of our goals, ultimately, is to determine the structural changes in the ligand binding domain of ERa that occur when calmodulin binds, and how these contribute to receptor activation. To date, we have not made as much progress towards this goal as we would have liked, but we have demonstrated that we can produce and purify this protein construct, and that it does bind to calmodulin (calmodulin affinity purification is the last step of the purification protocol). These studies are continuing with the goal of using this protein for NMR spectroscopy studies of the structural changes that occur in ERa when calmodulin binds and how these contribute to ERa activation.
- *The calmodulin binding region(s) of ERa reside(s) in amino acid residues 241-320*. Using an affinity tagged 241-320 (hinge region of ERa) construct ($Trx\text{-}ERA_{241-320}$), we demonstrated high affinity, calcium dependent binding to calmodulin.
- *A high affinity, calcium-dependent calmodulin binding site of ERa is further localized to residues 287-311*. Using a peptide synthesized by solid-phase methods ($HERA_{287-311}$), we have demonstrated a high affinity, calcium-dependent binding of calmodulin to residues 287-311 of ERa. The amino acid sequence represents a non-canonical calmodulin binding region, but nevertheless shares many of the properties of traditional high affinity calmodulin binding sites (basic, amphiphilic, probably helical).
- *A shorter region of ERa ($HERA_{295-311}$) does not represent the full calmodulin binding region*. An independent investigation by another research group has suggested that residues 295-311 of ERa constitute the calmodulin binding region. Our results indicate that this is incorrect. Firstly, our fluorescence results unequivocally demonstrate that W292 is buried in a hydrophobic pocket of one of the globular domains of calmodulin (this residue is not present in the shorter peptide). Secondly, we have compared directly the relative affinities of the longer and shorter peptides, and the shorter peptide clearly displays a much lower affinity for calmodulin.
- *An additional, low affinity calmodulin binding region includes residues 241-273 of ERa*. We have determined that a second, lower affinity calmodulin binding region of ERa is localized to regions 241-273 (hinge region) of ERa. We are unsure currently whether this represents a physiologically relevant site or simply non-specific binding. However, an additional site could assist in maintaining a high local concentration of calmodulin for ERa activation.
- *The low affinity calmodulin binding region that includes 241-273 of ERa is non-specific*. Control experiments indicate that this region binds to CaM if the amino acid sequence is randomized, and is therefore not sequence specific but dependent only on the composition of the amino acids comprising the domain.
- *The high affinity calmodulin binding region (287-311) of ERa contains three residues subject to posttranslational modifications that can potentially mediate calmodulin affinity*. Residues S305, K302 and K303 of ERa have all been shown to be posttranslationally modified (the serine phosphorylated, the lysines acetylated). Calmodulin binds to basic (and amphiphilic)

domains, so increasing the negative charge in this region (phosphorylating the serine) or decreasing the positive charge (acetylating the lysines) would be expected to decrease the affinity for calmodulin. Thus, it appears that nature has chosen to mediate ERa activation by attenuating calmodulin affinity by posttranslational modification.

- *The interaction of the ERa peptide ($HERA_{287-311}$) with calmodulin results in structural changes in calmodulin that suggest that the crowding from two bound peptides limits the structural collapse of calmodulin around the bound peptides and that unusual structural changes are occurring in the C-terminal binding pocket*. Using NMR spectroscopy, we have determined how the chemical shifts of calmodulin change when the ERa calmodulin binding peptide binds. The changes indicate that, compared to complexes of calmodulin with other high affinity targets, the complex of calmodulin with the ERa peptide is extended rather than collapsed. We suggest that this is due to crowding from the two bound peptides. We also observed some large, notable and atypical chemical shift changes in the C-terminal globular domain of calmodulin suggesting some novel structural attributes. We are in the process of determining a high resolution structure of this complex using NMR spectroscopy.
- *The calmodulin binding region of ERa ($HERA_{287-311}$) adopts both helical structure and random coil structure when bound to calmodulin*. Most calmodulin binding regions of target proteins adopt (basic, amphiphilic) helical character when they bind to calmodulin. The ERα peptide is no exception. However, in contrast to canonical CaM binding regions, a significant population of extended structure is also adopted (random coil). In the absence of calmodulin, circular dichroism indicates a total lack of helical structure, but the peptide clearly adopts some helical structure when bound to calmodulin.
- *Calmodulin binds to raloxifene*. To our knowledge, this is the first demonstration of the fact that CaM can bind to antiestrogens used for breast cancer therapies other than tamoxifen and hydroxytamoxifen.
- *Oxidation of the methionine residues in calmodulin results in the inability of calmodulin to bind to tamoxifen, hydroxytamoxifen and raloxifene*. It has been conjectured that the beneficial consequences of tamoxifen therapy for estrogen dependent breast cancers results from the binding of tamoxifen to calmodulin, which inhibits the ability of calmodulin to activate the receptor. Under conditions of high oxidative stress in breast cancer tissues, if the methionine residues in calmodulin are oxidized, tamoxifen no longer binds to calmodulin. This could be one mechanism by which antiestrogen resistance could develop.
- *The inability of oxidized calmodulin to bind tamoxifen/hydroxytamoxifen/raloxifene is due to altered polarity of the tamoxifen binding sites on calmodulin*. Control experiments show that replacement of all methionine residues in calmodulin with leucine does not substantially alter tamoxifen binding. Therefore, the polarity changes induced by oxidation of the methionine residues in calmodulin to methionine sulfoxide most likely is the cause of the results observed.
- *The complex of CaM with hydroxytamoxifen is more amenable to NMR characterization than the CaM*-TAM complex. Apparently, the very poor aqueous solubility of tamoxifen results in the inability to completely saturate the TAM binding sites on CaM, resulting in some conformational averaging in the NMR spectra. However, hydroxytamoxifen is slightly more soluble, resulting in compelte saturation. Thus, the complex of CaM with OHTAM is somewhat more amenable to high resolution characterization by NMR than the CaM-TAM complex.
- *Oxidation of CaM does not abrogate binding to ERa*. Our results indicate that oxidation of the methionine residues in CaM decreases the affinity of CaM for $HERA_{287-311}$, but not to a limited degree. The results suggest that CaMox most likely can still activate ERa, thus, oxidative stress most likely cannot eliminate activation of ERa by CaM. This is a key result, and suggests that blocking ERa activation by CaM with TAM (or its metabolites) can be reversed by oxidative stress, and therefore could be a component of oxidative stress induced antiestrogen resistance.

REPORTABLE OUTCOMES:

Abstracts/Presentations (cumulative):

We are currently completing some of the studies for two journal submissions that include results based on work funded by this CDMRP award. Results of these studies have been presented in a poster presentation at local/regional and national/international scientific meetings/conferences and are scheduled currently for presentation at one national/international conference this summer (full abstracts for these presentations appear in the "APPENDICES" section below):

Savannah J. Johnson, John A. Galdo, Marie E. Cross, Madeline C. Elliott, Ramona J. Bieber Urbauer and Jeffrey L. Urbauer (2007) *The Interaction of Calmodulin with Estrogen Receptor Alpha*. Southeast Regional Meeting of the American Chemical Society, October 24-27, Greenville, SC.

Ramona J. Bieber Urbauer, Carrie E. Jolly, Savannah J. Johnson, John Galdo, Madeline Elliott, Michael Nooromid and Jeffrey L. Urbauer (2008) *Calmodulin mediated estrogen receptor alpha activation and antiestrogen resistance*. 22nd Annual Symposium of the Protein Society, July 19-23, San Diego, CA.

Ramona J. Bieber Urbauer, Carrie E. Jolly, Savannah J. Johnson, John A. Galdo, Marie E. Cross, Madeline C. Elliott and Jeffrey L. Urbauer (2008) *Mechanistic Basis of Calmodulin Mediated Estrogen Receptor Alpha Activation and Antiestrogen Resistance*. Era of Hope 2008 Meeting, June 25-28, Baltimore, MD.

Ramona J. Bieber Urbauer, Carrie E. Jolly, Savannah J. Johnson, John A. Galdo, Marie E. Cross, Madeline C. Elliott, Michael Nooromid and Jeffrey L. Urbauer (2008) *Estrogen Receptor Alpha Activation by Calmodulin*. 23nd Annual Symposium of the Protein Society, July 25-29, Boston, MA.

Education/Training/Employment/Research Opportunities (cumulative):

To date, five undergraduate students, from both UGA, neighboring institutions, and institutions outside of the Southeast, have participated in, or are currently participating in, this project in my laboratory and have received important scientific training both in basic protein biochemistry and in cancer biology. These students (listed below) are all pursuing professional careers in science and medically related fields.

I'll also note that five of these students are co-authors of at least one of the presentations listed above and most, if not all, will be co-authors on planned journal submissions.

--Madeline Elliott (Honors Program, UGA), attending Mercer University School of Medicine (Fall, 2009)
--Michael Nooromid (Honors Program, UGA), attending New York University School of Medicine (Fall, 2009)
--John Galdo (UGA), in pharmacy school (The University of Georgia)
--Marie Cross (UGA), attending College of Dental Medicine, The Medical University of South Carolina
--Aisha Mahmood (UGA), undergraduate Honors Student, The University of Georgia
--*Noelle Cheung (Carnegie Mellon University), graduate school or medical school

--*Leah Cho (Denver University), graduate school or medical school
--**Savannah Johnson (Piedmont College), attending graduate school, Emory University Department of Chemistry

*Noelle and Leah were undergraduate participants in the SURO (Summer Undergraduate Research Opportunity) program in the Chemistry Department at UGA (summer, 2008).
**Savannah was a participant in the SURO (Summer Undergraduate Research Opportunities) program in the Department of Chemistry at UGA during the summer of 2007.

Likewise, a Postdoctoral Researcher in my laboratory, Dr. Carrie Jolly, worked on various aspects of this project during her 20 month stay. Dr. Jolly has since moved on to Montana with her family, where she is now employed as a postdoctoral researcher at the National Institutes of Health, National Institute of Allergy and Infectious Diseases, at the Rocky Mountain Laboratories.

In August, a new undergraduate student, Ashley Itua, will be initiating her undergraduate research working on this project.

CONCLUSION and "SO-WHAT" section (cumulative):

Knowledge of the precise binding site or sites for CaM on ERa is essential ultimately for understanding, from a structural and mechanistic perspective, how calmodulin binding to ERa activates ERa. Because it is apparent that the C-terminal end of the CaM binding domain is part of the ligand binding domain of ERa, CaM binding most likely affects the structure of the ligand binding domain, and therefore the current mechanisms for E2 activation and antiestrogen inactivation are at least incomplete if not incorrect. Furthermore, recent studies have "localized" the CaM binding domain to a short section of the hinge region, and we have demonstrated that this is incorrect. In addition, we have found what appears to be a second, lower affinity CaM binding site in the N-terminal region of the hinge, which appears to be Ca^{2+}-independent. Allthough this site apparently is non-specific (not sequence specific), this could have important implications for CaM binding and ERa activation, and could represent a mechanism to improve affinity of CaM for ERa without the need for sequence specificity.

CaM binding domains of dozens of proteins activated by CaM are most always basic, amphiphilic, and adopt helical structure when bound to CaM. The high affinity site that we have localized on ERa appears similar in these respects, except that only part of the binding region adopts helical structure, and the remainder apparently does not (random coil). The more rigid helical structure often adopted by these domains is integral to the mechanism of activation, and often represents the first, obligatory step in the activation process. In this case, perhaps the activation process only necessitates partial helical character. Thus, it is critical to understand these structural changes in order to define mechanistically how activation occurs. In the same vein, the structural changes that occur in CaM when it binds to these domains is also important. These changes may be very large, as is the case for binding to the MLCK (discussed above), or smaller, as is the case for ERa. This is important, as it signifies that a large scale "collapse" of CaM around the high affinity domain in ERa is less dramatic, and reflects fundamantal structural attributes associated with the activation process, In the case of ERa, we suspect that these more modest structural changes result from the fact that CaM can bind to two CaM binding domains of ERa simultaneously, and that the crowding resulting from the presence of two peptides does not permit a dramatic collapse, as is observed for MLCK (for instance).

Our hypothesis is that CaM and oxidative stress are integral to the process of antiestrogen development. Our rationale is as follows. It has been suggested that tamoxifen (TAM) binding to CaM blocks CaM activation of ERa, and therefore contributes positively to

tamoxifen therapy for estrogen dependent breast cancer. The oxidative stress level (and levels of reactive oxygen species) in breast cancer tissue is high, and because the methionine residues in CaM are easily oxidized, we tested whether oxidized CaM could still bind TAM. It does not. Furthermore, hydroxytamoxifen (one of the active metabolites of TAM) and raloxifene (another antiestrogen, but chemically distince from TAM and metabolites) display the same behavior. If oxidized CaM can still bind and activate ERa, then TAM/OHTAM/raloxifene would not be able to as effectively inhibit CaM activation of ERa when CaM is oxidized. Thus, this would be a mechanism for development of antiestrogen resistance. Furthermore, we know also know that CaM oxidation does not eliminate binding to ERa, indicating that CaMox most likely can still activate ERa, thus, oxidative stress most likely cannot eliminate activation of ERa by CaM. This is a key result, and suggests that blocking ERa activation by CaM with TAM (or its metabolites) can be reversed by oxidative stress, and therefore could be a component of oxidative stress induced antiestrogen resistance.

Overall, our studies to date have been very successful and informative. We have established a very firm foundation for continuing the work that we originally proposed.

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2. Schwabe, J. W., Chapman, L., Finch, J. T., and Rhodes, D. (1993) The crystal structure of the estrogen receptor DNA-binding domain bound to DNA: how receptors discriminate between their response elements, *Cell 75*, 567-578.
3. Hoeflich, K. P., and Ikura, M. (2002) Calmodulin in action: diversity in target recognition and activation mechanisms, *Cell 108*, 739-742.
4. Yap, K. L., Kim, J., Truong, K., Sherman, M., Yuan, T., and Ikura, M. (2000) Calmodulin target database, *J Struct Funct Genomics 1*, 8-14.
5. Gallo, D., Jacquot, Y., Laurent, G., and Leclercq, G. (2008) Calmodulin, a regulatory partner of the estrogen receptor alpha in breast cancer cells, *Mol Cell Endocrinol*.
6. Gallo, D., Jacquemotte, F., Cleeren, A., Laios, I., Hadiy, S., Rowlands, M. G., Caille, O., Nonclercq, D., Laurent, G., Jacquot, Y., and Leclercq, G. (2007) Calmodulin-independent, agonistic properties of a peptide containing the calmodulin binding site of estrogen receptor alpha, *Mol Cell Endocrinol 268*, 37-49.
7. Wu, X., Hawse, J. R., Subramaniam, M., Goetz, M. P., Ingle, J. N., and Spelsberg, T. C. (2009) The tamoxifen metabolite, endoxifen, is a potent antiestrogen that targets estrogen receptor alpha for degradation in breast cancer cells, *Cancer Res 69*, 1722-1727.
8. Lim, Y. C., Li, L., Desta, Z., Zhao, Q., Rae, J. M., Flockhart, D. A., and Skaar, T. C. (2006) Endoxifen, a secondary metabolite of tamoxifen, and 4-OH-tamoxifen induce similar changes in global gene expression patterns in MCF-7 breast cancer cells, *J Pharmacol Exp Ther 318*, 503-512.
9. Lim, Y. C., Desta, Z., Flockhart, D. A., and Skaar, T. C. (2005) Endoxifen (4-hydroxy-N-desmethyl-tamoxifen) has anti-estrogenic effects in breast cancer cells with potency similar to 4-hydroxy-tamoxifen, *Cancer Chemother Pharmacol 55*, 471-478.

APPENDICES:

Abstracts (cumulative):

Savannah J. Johnson, John A. Galdo, Marie E. Cross, Madeline C. Elliott, Ramona J. Bieber Urbauer and Jeffrey L. Urbauer (2007) *The Interaction of Calmodulin with Estrogen Receptor Alpha*. Southeast Regional Meeting of the American Chemical Society, October 24-27, Greenville, SC.

Estrogen dependent breast cancers require the transcriptional activation activity of the estrogen receptor alpha (ERa). These account for approximately 70% of all breast cancers. In response to estrogen binding, a set of genes is activated by ERa. This facilitates growth and propagation of the cancer cells. The important calcium-binding signaling protein calmodulin (CaM) binds ERa, and, recently, it was demonstrated that CaM is essential for activation of ERa transcriptional activity. Here we present progress towards defining the CaM binding region of ERa and the structural changes in CaM upon interaction with ERa, and towards understanding potential cellular mechanisms for mediating activation of ERa by CaM. As a first step towards elucidating the mechanism of ERa activation by CaM, we have produced a segment of the ERa protein (residues 241-320) as a thioredoxin fusion and demonstrated binding to CaM. A short, 25 amino acid section of this segment of ERa, suspected to comprise the CaM binding sequence, was produced, as were three derivatives containing modifications known to occur naturally. Fluorescence titration experiments demonstrated that these peptides all bind to CaM, and they all bind with rare 2:1 (peptide:CaM) stoichiometries. Their relative binding affinities are consistent with established principles for CaM interactions with target domains. The complex of CaM with the wild-type peptide was studied using NMR spectroscopy. The chemical shifts of CaM were assigned using triple resonance methods. The changes in chemical shifts upon peptide binding suggest an altered binding mode relative to typical complexes of CaM with target peptides.

Ramona J. Bieber Urbauer, Carrie E. Jolly, Savannah J. Johnson, John Galdo, Madeline Elliott, Michael Nooromid and Jeffrey L. Urbauer (2008) *Calmodulin mediated estrogen receptor alpha activation and antiestrogen resistance*. 22nd Annual Symposium of the Protein Society, July 19-23, San Diego, CA.

Estrogens and estrogen receptor alpha (ERa) are central to estrogen-dependent breast cell carcinoma induction and proliferation. ERa is the principal target for systemic endocrine/antiestrogen therapy, underscoring its biological relevance and medical importance. Recently, it was established that calcium-dependent activation by calmodulin (CaM) is essential for estrogen-dependent ERa activity and that the active species is the CaM-ERa complex. CaM also binds tightly to antiestrogens, including the most widely used chemotherapeutic agent for estrogen-dependent breast cancers, tamoxifen (TAM). The therapeutic effects of antiestrogens are indicated to be due, in part, to CaM antagonism. Our objectives include establishing the molecular mechanism whereby CaM activates estradiol-dependent ERa transcription and defining the role of oxidative stress in mediating CaM-ERa and CaM-antiestrogen interactions. We have localized the CaM binding region of ERa to a 25 amino acid segment in the ERa hinge region and have initiated NMR spectroscopy studies to determine the structure of the complex of CaM with this CaM binding region. Based on chemical shift changes, the collapse of CaM around the ERa CaM binding domain is much less dramatic than observed for complexes of CaM with prototypical binding domains, with relatively large structural changes occur in the C-terminal domain of CaM. These results suggest that CaM bound to ERa is more extended structurally compared to typical CaM complexes and signify important structural changes in the C-terminal binding pocket of CaM. Oxidation of the methionine residues in CaM eliminates

binding to TAM and hydroxy-TAM. TAM binding to mutant CaM where all methionine residues are replaced by leucine is unaffected by the leucine substitutions. Methionine oxidation results in polarity changes that decrease the affinity of CaM for hydrophobic drugs. The results are important for a comprehensive understanding of CaM activation of ERa and the link between oxidative stress and development of antiestrogen resistance.

Ramona J. Bieber Urbauer, Carrie E. Jolly, Savannah J. Johnson, John A. Galdo, Marie E. Cross, Madeline C. Elliott and Jeffrey L. Urbauer (2008) *Mechanistic Basis of Calmodulin Mediated Estrogen Receptor Alpha Activation and Antiestrogen Resistance*. Era of Hope 2008 Meeting, June 25-28, Baltimore, MD.

Estrogens and estrogen receptor alpha (ERa) are central to estrogen-dependent breast cell carcinoma induction and proliferation. ERa is the principal target for systemic endocrine/antiestrogen therapy, underscoring its biological relevance and medical importance. Recently it has been established that calcium-dependent activation by calmodulin (CaM) is essential for estrogen-dependent ERa activity, and that the active species is the CaM-ERa complex (Li, L., Li, Z., and Sacks, D. B. (2005) *J Biol Chem 280*, 13097-104.). CaM also binds tightly to antiestrogens, including the most widely used chemotherapeutic agent for estrogen-dependent breast cancers, tamoxifen (TAM). The therapeutic effects of antiestrogens, like those of other CaM antagonists, are indicated to be due, in part, to the direct interaction with CaM.

Oxidative stress – estrogen-induced oxidative stress and constitutive oxidative stress – is indicated in estrogen-dependent breast cancer tissues. Oxidative stress is also implicated as mediating development of resistance of breast cancers to antiestrogens. TAM is also implicated in inducing a potent oxidative stress response in breast cancer tissue. It has been demonstrated in other oxidatively stressed tissues (senescent brain), that increased levels of reactive oxygen species and the failure of cellular repair mechanisms conspire to cause accumulation of oxidized CaM species (where one or more of the nine methionine residues are oxidized to the sulfoxides), altering intracellular calcium homeostasis. Oxididation of CaM can reduce its ability to activate some target proteins, without necessarily reducing binding affinity. Because there are nine methionine residues in CaM, most of which interact with the CaM binding domains of target proteins, the effects of oxidation can be specific for particular methionine residues.

Our objectives include establishing the molecular mechanism, including the structural details, by which CaM activates estradiol-dependent ERa transcription, and defining the role of oxidative stress in mediating CaM-ERa and CaM-antiestrogen interactions. Towards these goals we have localized the CaM binding region of ERa to a 25 amino acid segment in the hinge region of ERa. We have initiated studies using NMR spectroscopy to determine the structure of the complex of CaM with this CaM binding region of ERa. To date, based on chemical shift changes, we have found the collapse of CaM around the ERa CaM binding domain is much less dramatic than observed for complexes of CaM with prototypical binding domains, and that relatively large structural changes occur in the C-terminal domain of CaM. Oxidation of the methionine residues in CaM eliminates binding to TAM and hydroxy-TAM. TAM binding to CaM with all methionine residues replaced by leucine is unaffected by the leucine substitutions. These results suggest that CaM bound to ERa is more extended structurally compared to typical CaM complexes and signify important structural changes in the C-terminal binding pocket of CaM. Oxidation of methionine residues in CaM results in polarity changes that decrease the affinity of CaM for hydrophobic drugs. The results will be important for a comprehensive understanding of the principles governing CaM activation of ERa and the link between oxidative stress and development of antiestrogen resistance, in order to aid in the design and development of a new pharmaceuticals to treat breast cancers.

Ramona J. Bieber Urbauer, Carrie E. Jolly, Savannah J. Johnson, John A. Galdo, Marie E. Cross, Madeline C. Elliott, Michael Nooromid and Jeffrey L. Urbauer (2009) *Estrogen*

Receptor Alpha Activation by Calmodulin. 23rd Annual Symposium of the Protein Society, July 25-29, Boston, MA.

The alpha isoform of the estrogen receptor (ERα) is the principal target for systemic endocrine/antiestrogen therapy for estrogen-dependent breast cancers. It has been known for some time that ERα is subject to calcium-dependent activation by calmodulin (CaM), and recently this interaction with CaM was demonstrated to be essential for activation. CaM also binds tightly to antiestrogens, including the most widely used chemotherapeutic agent for estrogen-dependent breast cancers, tamoxifen (TAM), and its metabolites. It has been suggested that therapeutic benefit of antiestrogens for estrogen-dependent breast cancers may derive partially from CaM antagonism.

We are interested in defining the molecular mechanism of CaM activation of ERα and establishing how oxidative stress might mediate the interactions of CaM with ERα and antiestrogens. Recently we localized the CaM binding region of ERα and initiated NMR studies to determine the solution structure of the complex of CaM with the ERα CaM binding region. Chemical shift changes indicate that CaM bound to ERα is more extended structurally compared to typical CaM complexes and identify important structural changes in the C-terminal binding pocket of CaM. The ERα CaM binding region adopts partial helical character upon CaM binding. Oxidation of the methionine residues in CaM eliminates binding to TAM, its metabolites and related hydrophobic antiestrogens, but these still bind to mutant CaM where all methionine residues are replaced by leucine. Oxidation reduces the affinity of CaM for ERα but does not eliminate it. Methionine oxidation results in polarity changes that decrease the affinity of CaM for hydrophobic drugs. The results are important for a comprehensive understanding of CaM activation of ERα and the link between oxidative stress and development of antiestrogen resistance.

CV:

A cv is attached, following the “Supporting Data” section

SUPPORTING DATA:

All figures are embedded in the text (above), along with their respective figure legends.

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EDUCATION

B.A.	Chemistry, *magna cum laude*, University of Nebraska-Lincoln, Lincoln, Nebraska, 1981 (Minors: Life Sciences, English)
Ph.D.	Chemistry, Department of Chemistry, University of Nebraska-Lincoln, Lincoln, Nebraska, 1987

POSTDOCTORAL

1988-1991	National Institutes of Health Postdoctoral Fellow, Department of Biochemistry and Institute for Enzyme Research, University of Wisconsin-Madison, Madison, Wisconsin (with W. W. Cleland)
1991-1992	Postdoctoral Associate, Department of Biochemistry and Institute for Enzyme Research, University of Wisconsin-Madison, Madison, Wisconsin (with W. W. Cleland)
1992-1995	Postdoctoral Associate, Department of Biochemistry, University of Illinois Urbana/Champaign, Urbana, Illinois (with A. Joshua Wand)

ACADEMIC APPOINTMENTS

1995	Research Scientist, Department of Biochemistry, University of Illinois Urbana/Champaign, Urbana, Illinois
1995-1998	Research Assistant Professor, Department of Chemistry and Center for Structural Biology, State University of New York, University at Buffalo, Buffalo, New York
1998-1999	Research Assistant Professor, Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania
1999-2003	Assistant Professor, Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas
2003-2005	Associate Professor, untenured, Department of Biochemistry and Molecular Biology and Department of Chemistry, University of Georgia, Athens, Georgia
2005-	Associate Professor, tenured, Department of Biochemistry and Molecular Biology and Department of Chemistry, University of Georgia, Athens, Georgia

HONORS / AWARDS

2002	Class of 2002 Favorite Biology Professor, University of Kansas
2001	Mortar Board National Senior Honor Society Outstanding Educator 2001, Torch Chapter, University of Kansas
2001	Finalist Award, Madison and Lila Self Faculty Scholar, University of Kansas
1988-1991	National Institutes of Health Postdoctoral Fellow, University of Wisconsin-Madison
1984	Phi Lambda Upsilon, Rho Chapter, University of Nebraska-Lincoln
1981	B. A., *magna cum laude*, University of Nebraska-Lincoln
1977-1978	David Scholarship, University of Nebraska-Lincoln

PROFESSIONAL AFFILIATIONS

1983-	American Association for the Advancement of Science
1983-	American Chemical Society
1988-	American Society for Biochemistry and Molecular Biology
1996-	Protein Society
2000-	Biophysical Society

Previous Funding

2000-2002 NSF, DBI-0088931 (PI)
A 600 MHz NMR Spectrometer for the University of Kansas
09/01/2000 – 08/31/2002
Direct Costs - $313,990 (two years)

2001 Higuchi Biosciences Center (co-investigator)
Stable Laser Source for Single Molecule and Two Photon Microscopy
02/19/2001
Direct Costs - $30,000

2001 KU Center for Research (co-investigator)
Advances in High Field and High Throughput NMR Analysis
11/05/2001
Direct Costs - $48,625

1997-1998 NIH/University of Colorado (PI for subcontract)
Subcontract from NIH 7 R01 GM50700
Structural Studies of the AsiA Protein
Direct Costs - $19,446 (two years)

1988-1991 NIH/NIGMS GM12511 (Awardee, National Research Service Award)
Mechanism of Enzymatic Oxidative Decarboxylations
07/01/1988 – 06/30/1991
Direct Costs - $56,000 (approximate, three years)

2002-2003 NIH/NCRR P20 RR16475/K-BRIN (PI for subproject)

Alginate Production/Virulence in Pseudomonas aeruginosa
07/01/2002 – 06/30/2003
Direct Costs - $25,000 (one year)

2002-2003 Higuchi Biosciences Center (PI)
Structural Biology of Virulence Regulation in Pseudomonas aeruginosa
07/01/2002 – 06/31/2003
Direct Costs - $30,000 (one year)

2001-2003 University of Kansas Center for Research (PI)
Initial Structural Characterization of Regulatory Proteins of Pseudomonas aeruginosa Involved in Production of Mucoid Exopolysaccharide and Other Virulence Factors
09/04/2001 – 09/03/2003
Direct Costs - $10,000 (two years)

2002 Madison and Lila Self Faculty Scholar Fund (PI)
Self Faculty Scholar Finalist Award
Structural and Functional Proteomics/Genomics of the Progression to Mucoidy and Virulence in Pseudomonas aeruginosa
Direct Costs - $5,000

2001-2003 American Heart Association 0120666Z (mentor/advisor)
Molecular Consequences of Oxidative Stress: Stability and Structure of an Oxidized Calmodulin/Target Protein Complex
07/01/2001 – 06/30/2003
Direct Costs - $77,622 (two years)
This is a postdoctoral fellowship awarded to Dr. Asokan Anbanandam, a postdoctoral researcher in my laboratory.

2003-2004 Higuchi Biosciences Center (PI)
HPLC Instrumentation for Biophysical Studies
02/19/2003 – 02/18/2004
Direct Costs - $25,000 (one year)

2003-2004 NIH/NIGMS, R01 GM058715 (co-investigator, PI for subcontract)
Single-Molecule Dynamics of Target Binding by Calmodulin
08/16/03 – 08/30/04 (subcontract)
Direct Costs – $21,695

2001-2005 NIH/NCI, R01 CA085917 (co-investigator)
Modulation of the Intercellular Junction Cadherin
06/01/2001 – 05/31/2005
Direct Costs - $700,000 (four years)

1999-2006 NIH/NIGMS, R01 GM54998 (PI)
Structure and Dynamics of AsiA and the AsiA-σ^{70} Complex
05/01/1999 - 04/31/2006
Direct Costs, $683,115 (five years)

2000-2006 NIH/NIA, R01 AG17996 (co-investigator)
Structural Basis for Altered Calcium Homeostasis During Aging
04/01/2000 - 03/31/2006
Direct Costs, $1,000,000 (four years)

2001-2006 NIH/NHLBI, R01 HL65524 (co-investigator)
Regulatory Mechanisms of Secretory Phospholipases A2
02/01/2001 – 01/31/2006
Direct Costs - $875,000 (four years)

2006 – 2008 NIH/NIAID, F32 AI065070 (Sponsor*)
Structure, Dynamics, and Function of AlgH
04/01/06 – 03/31/08
Direct Costs - $85,753 (two years)
*This is a Postdoctoral Fellowship (NIH NRSA) to Dr. Aaron Cowley, a postdoctoral researcher in my laboratory. Dr. Cowley only used about 6 months of this support before finding a job in industry.

2005-2007 Georgia Cancer Coalition (PI)
Activation of Estrogen Receptor Alpha by Calmodulin
11/01/2005 – 04/28/2007
Direct Costs – $34,200 (one year, extended)

Current Funding

2007-2011 NIH/NIGMS, R01 GM54998 (PI)
Structure and Dynamics of AsiA and the AsiA-σ^{70} Complex
09/01/2007 - 08/31/2011
Direct Costs, $632,000 (four years)

2007-2010 DOD/CDMRP, BC061820 (PI)
Mechanistic Basis of Calmodulin Mediated Estrogen Receptor Alpha Activation and Antiestrogen Resistance
01/01/2007 – 12/31/09
Direct Costs - $300,000 (three years)

2006-2009 NIH/NIAID, R21 AI070933 (PI)
Regulating Microbial Biofilm Formation: A Novel Prokaryotic Multi-Protein Complex
06/01/06 – 05/31/09
Direct Costs – $275,000 (three years)

PENDING FUNDING

2009-2013 NSF
Structural Basis for Interfacial Activation of Secreted Phospholipase A2 Enzymes

02/01/2009 – 01/31/2013
Direct Costs - $944,266 (four years)

2009-2013 NSF
Molecular Mechanisms of Secreted Phospholipase A2 Enzymes
08/01/2009 – 07/31/2013
Direct Costs - $940,000 (four years)

OTHER FUNDING ACTIVITIES

2002-2007 NIH/NCRR P20RR017708 (COBRE Program) (Faculty Mentor/Resource Faculty)
Protein Structure and Function
11/01/02 – 10/31/07
Direct Costs - $8,163,476 (approximate, 5 years)
My role was as 'scientific mentor' for Dr. Susan Egan, one of the recipients of research funding from this award, and also as NMR 'resource faculty'. I received some salary compensation from this award.

2002-2007 NIH/NIGMS T32GM008359 (potential preceptor)
Pharmaceutical Aspects of Biotechnology Training
07/01/2002 – 06/30/2007
Direct Costs - $1,250,000 (five years)
This is a predoctoral training grant.

2002-2007 NIH/NIGMS 1K12GM063651-01A1 (potential preceptor)
The University of Kansas Haskel University IRACDA‡ Project
08/01/2002 – 07/31/07
Direct Costs - $2,300,000 (five years)
‡Institutional Research and Academic Career Development Award
This is a postdoctoral training grant.

2002-2007 NIH/NIGMS P41GM66326 ("major user")
900 MHz NMR Spectrometer Biomolecular Investigations
07/01/2002 – 06/30/2007
Direct Costs - $3,699,620 (five years)
This proposal is for 900 MHz NMR instrumentation at the National Magnetic Resonance Facility at Madison (University of Wisconsin-Madison).

2003-2008 NIH/NIGMS P41GM068928 ("major user")
Purchase of 900 MHz Spectrometer
07/15/2003 – 06/30/2008
Direct Costs – (unknown) (five years)
This proposal is for 900 MHz NMR instrumentation at the Rocky Mountain Regional NMR Center (University of Colorado Health Sciences Center).

PATENTS

"*Apparatus and Method for High Pressure NMR Spectroscopy*" (Nov. 2, 1999)
Inventors: A. Joshua Wand, Mark R. Ehrhardt, Jeffrey L. Urbauer
Assignee: Research Foundation of the State University of New York

TEACHING

University of Georgia:

Fall, 2003: CHEM/BCMB 4190/6190/8189 (*Introductory NMR*, 100%)		11[Ω]	3[Σ]	1.22*
Spring, 2004; CHEM/BCMB 8190	(*Biomolecular NMR, 25%)*	6	3	n/a
Fall, 2004: CHEM/BCMB 4190/6190/8189 (*Introductory NMR*, 100%)		9	3	1.00
Fall, 2004: CHEM/BCMB 8110 (*Protein Structure and Function*, 40%)		8	3	n/a
Spring, 2005: CHEM/BCMB 8190	(*Biomolecular NMR, 25%)*	6	3	n/a
Fall, 2005: CHEM/BCMB 4190/6190/8189 (*Introductory NMR*, 100%)		12	3	1.50
Spring, 2006: CHEM/BCMB 8190	(*Biomolecular NMR, 25%)*	5	3	n/a
Spring, 2006: CHEM 8220 (*Phys. Meth. in (Bio)Inorg. Chem*., 12%)		8	3	n/a
Fall, 2006: CHEM/BCMB 4190/6190/8189 (*Introductory NMR*, 100%)		12	3	1.50
Fall, 2006: BCMB 8060	(*BCMB seminar*, 33%)	43	1-2	n/a
Spring, 2007: BCMB 8060	(*BCMB seminar*, 33%)	41	1-2	n/a
Spring, 2007: CHEM/BCMB 8110 (*Protein Structure and Function*, 35%)		6	3	1.25
Spring, 2008: CHEM/BCMB 8190	(*Biomolecular NMR, 25%)*	5	3	n/a
Spring, 2008: CHEM 8220 (*Phys. Meth. in (Bio)Inorg. Chem*., 12%)		8	3	n/a
Fall, 2008: CHEM/BCMB 4190/6190/8189 (*Introductory NMR*, 100%)		11	3	1.00
Fall, 2008: BCMB 8060	(*BCMB seminar*, 33%)	32	1-2	n/a
Spring, 2009: BCMB 8060	(*BCMB seminar*, 33%)	30	1-2	n/a

Other: Fall, 2008: two lectures in CHEM 3300 (lectures on NMR spectroscopy)

[Ω]number of students
[Σ]credits/hours
*average student evaluation: "*The instructor is an excellent teacher: strongly agree (1), agree (2), neutral (3), etc*."
n/a: not applicable – multiple instructors (individual instructors not evaluated), seminar course, etc.

University of Kansas:

Fall, 2000:	Biology 658 (*Biochemistry I*[†], 50%)	67 students	3[Σ]	4.66*
	Biology 750 (*Advanced Biochemistry*[‡], 30%)	23 students	3	4.75
Spring, 2001:	Biology 600 (*Introductory Biochemistry*[¶], 50%)	103 students	4	4.62
Fall, 2001:	Biology 658 (*Biochemistry I*, 50%)	67 students	3	4.94
	Biology 750 (*Advanced Biochemistry*, 30%)	20 students	3	4.89
Spring, 2002:	Biology 600 (*Introductory Biochemistry*, 50%)	109 students	4	4.64
Fall, 2002:	Biology 658 (*Biochemistry I*, 50%)	68 students	3	4.79
	Biology 750 (*Advanced Biochemistry*, 50%)	9 students	3	4.89
	Biology 901 (*Graduate Seminar*[§], 100%)	7 students	1	5.00

Spring, 2003: Biology 701 (*Introductory Bioinformatics*, 8-10%)34 students 3 NA

Other: Fall, 1999: three lectures in Biochemistry 750 ("*Isotope Effects on Enzymatic Reactions*", "*Principles of NMR of Biomolecules*", "*Solution Protein Structure: Practical Examples*"), one lecture in Biochemistry 918[Δ], ("*Motional Properties of Proteins using NMR*")
Spring, 2001: two lectures in Biology 420[π] (various topics)
Fall, 2001: one lecture in Biochemistry 918 ("*Practical Heteronuclear NMR*"), one lecture in Biology 419[◊] ("*Transcription Initiation and AsiA*")
Fall, 2002: one lecture in Biology 419 ("*Structural Basis of Prokaryotic Transcription Initiation*")

*average student evaluation; "How would you rate this instructor" (4.5-5.0="A", 4.0-4.5= "B", etc.)
[Σ]credits/hours
[†]first course of a two semester series for undergraduate biochemistry majors
[‡]graduate course for Molecular Biosciences graduate students
[¶]single semester undergraduate course for non-majors, pre-meds, etc.
[§]*Graduate Seminar in Biochemistry and Biophysics*, required seminar course for all advanced biochemistry degree candidates
[Δ]*Modern Biochemical and Biophysical Methods*, required course for all advanced biochemistry degree candidates
[π]*Seminar in Biochemistry*, required course for all undergraduate Biochemistry majors
[◊]*Advanced Biology Seminar*, required for all biology Honors students
NA Not available (team-taught by several instructors, students did not provide separate instructor evaluations)

Teaching/Mentoring Recognition

Nominee, Class of 2003 Favorite Biology Professor, University of Kansas
Awardee, Class of 2002 Favorite Biology Professor, University of Kansas
Annual, single recipient from approximately 60 faculty in the Division of Biological Sciences: Awarded by graduating seniors of the Division of Biological Sciences "in recognition of and appreciation for outstanding teaching and exemplary service to undergraduate students".
Awardee, Mortar Board National Honor Society Outstanding Educator 2001, Torch Chapter, University of Kansas
Annual, 4 or 5 recipients, University wide: Awarded by the members of the Torch Chapter of the Mortar Board National Senior Honor Society for "their devotion to academia, teaching style, accessibility, knowledge of their subject and other special qualities".
Nominee, Byron A. Alexander CLAS Graduate Mentor Award, College of Liberal Arts and Sciences, 2002, University of Kansas
Nominee, Outstanding Mentor Award, Graduate and Professional Association, Graduate School, 2002, University of Kansas
Nominee, Class of 2001 Favorite Biology Professor, University of Kansas

SERVICE

Professional

Manuscript reviewer: *Journal of Physical Chemistry, Biochemistry, Journal of the American Chemical Society, Biophysical Journal, Journal of Biomolecular NMR, Journal of Biological Chemistry, Protein Science, Journal of Molecular Biology, Protein Expression and Purification*
Best Poster Awards Judging Committee, 22nd Annual Symposium of the Protein Society, 2008
Grant proposal reviewer, NSF, 2008
Best Poster Awards Judging Committee, 21st Annual Symposium of the Protein Society, 2007
Grant proposal reviewer, NSF, 2007
Grant proposal reviewer, NSF, 2006
Grant proposal reviewer, Agency for Science, Technology and Research, Biomedical Research Council (Singapore), 2006
Grant proposal reviewer (ad hoc), NIH/NIGMS Molecular Structure and Function C (MSFC), 2005
Grant proposal reviewer, American Chemical Society Petroleum Research Fund, 2004
Grant proposal reviewer, Utah State University Community/University Research Initiative (CURI), 2003
Kansas City Area Life Sciences Initiative (KCALSI) Research Grant Review Committee, 2002
Chair, session on Membrane Proteins, 16th Annual Symposium of the Protein Society, 2002
Active Member, American Chemical Society Legislative Action Network, 2001-present
-2003 ACS Legislative Action Network Honor Roll
-2004 ACS Legislative Action Network Honor Roll
("*The Honor Roll recognizes ACS members who have demonstrated a deep commitment to the promotion of legislation and policy that advances science*.")

University of Georgia

Departmental:
Chemistry Department:
Graduate Admissions Committee (Fall 2003 – present)
New Media Committee (Fall 2003 – 2004)
Graduate Recruiting Committee (Fall 2004 – present)
Executive Committee (Fall, 2004 – Fall, 2006)
Departmental Faculty (Organic) Search Committee (Fall, 2005 – Spring, 2006)
Departmental Search Committee, NMR manager (2006)
SURO Research Mentor, (2007, 2008)
Graduate Student Recruiting at SERMACS; 2004, 2005, 2006, 2007, 2008

Department of Biochemistry and Molecular Biology
Head of Seminar Committee (Fall, 2008 – present)
Seminar Committee (Fall, 2006 – present)
Departmental Faculty Search Committee (Fall, 2007 – Spring, 2008)

Other:
University Representative on the Governing Council for the Southeast Collaboratory for High-Field Biomolecular NMR (Fall 2003 – 2007)
Georgia Research Alliance / New Projects Focus Group coordinator for the Southeast Collaboratory for High-Field Biomolecular NMR (Summer 2004 – 2007)
CURO Honors Research Mentor (2006 - present)
Mentor, Honors Faculty Mentor Program (2007 - present)

University of Kansas

Departmental: Departmental Graduate Admissions and Policy Committee, 2002-2003

Departmental Seminar Committee, 2000-2003
Departmental Honors and Awards Committee, 2000-2003
Newmark Lecture Committee, 2000-2003
Departmental Committee to Establish Guidelines for Evaluation of Teaching and Service Contributions, 2002-2003
Biochemistry/Biophysics Section representative for the Molecular Biosciences Library Fund review, 2001
Departmental Search Committee, Structural Biologist search, 2000-2001
Departmental Search Committee, Structural Biologist search, 2001-2002
Departmental Search Committee, Bioinformatics Specialist search, 2002
Departmental Search Committee, Bioinformatics Specialist search, 2003
Search Committee, Chemistry Department, Bioinorganic Faculty search, 2003

Division: Chair, Division of Biological Sciences Honors Committee, 2002-2003
Division of Biological Sciences Honors and Awards Committee, 2000-2002

University: Assistant Director, Bioinformatics Initiative, Kansas BRIN, 2000-2003
Mentor, University Scholars Program, 2002-2003
Biological Research Service Laboratory Oversight Committee, 1999-2003

THESIS/ORAL EXAMINATION COMMITTEES

*****Chair or Advisor [†]Temporary committee member

Current

Wendy Nkari	Dept. of Chemistry, UGA
Laura Pallas	Dept. of Food Science and Technology, UGA
Jodi Hadden	Dept. of Chemistry, UGA

Previous

[†]Dr. Sandra Kinnear	Dept. Biology (State University of New York at Buffalo)
[†]Dr. Scott Walsh	Dept. Biochemistry and Biophysics (Univ. Pennsylvania)
Dr. Junichi Komoto	Dept. Molecular Biosciences
Dr. Yafei Huang	Dept. Molecular Biosciences
Dr. Ray Hein	Dept. Molecular Biosciences
[†]Dr. Lisa Kueltzo	Dept. Pharmaceutical Chemistry
Dr. Aaron Cowley	Dept. of Chemistry
*Dr. Ryan Bartlett	Dept. Molecular Biosciences
*Dr. Joshua Gilmore	Dept. Molecular Biosciences (KU)
Dr. Laura Lucas	Dept. of Chemistry
Colin Taylor, M.S.	Dept. Molecular Biosciences
Shreya Shaw, M.S.	Dept. Molecular Biosciences
Safet Hatic, M.S.	Dept. Molecular Biosciences
Farhana Afroz, M.S.	Dept. Chemistry
Martha Healy, M.S.	Dept. Medicinal Chemistry
Brett Hronek, M.S.	Dept. Molecular Biosciences
Kai Zheng, M.S.	Dept. Molecular Biosciences
[†]Laura Morris, M.S	Dept. Chemistry (UGA)
[†]Dr. Jennifer Whittier	Dept. Molecular Biosciences
Dr. Curt Boshek	Dept. Molecular Biosciences
Dr. Jason Wickstrom	Dept. Molecular Biosciences

MengMeng Wang	Dept. Molecular Biosciences
Sirisha Kodeboyina	Dept. Molecular Biosciences
Hardeep Samra	Dept. Molecular Biosciences
Amanda (Devoy) Harrington	Dept. Molecular Biosciences
Greg Osterhaus	Dept. Molecular Biosciences
†Ross Grigsby	Dept. Molecular Bioscience
Dr. Melanie Priestman	Dept. Medicinal Chemistry
Visnja Jevtic	Dept. Molecular Biosciences
*****Feng He	Dept. Molecular Biosciences
*Ana Kolin	Dept. Molecular Biosciences
*Dr. Roma Kenjale	Dept. Molecular Biosciences
Dr. Qinyi Cheng	Dept. Molecular Biosciences
Dr. Brian Slaughter	Dept. Chemistry
Dr. Chris Phillips	Dept. Molecular Biosciences
*****Dr. Jamie Zerbe	Dept. Molecular Biosciences
Kathy Meneely	Dept. Molecular Biosciences
†*Sami Tuomivaara	Dept. of Biochemistry and Molecular Biology, UGA
†*Jihye Shim	Dept. of Chemistry, UGA
Dr. Hsiau-wei (Jacques) Lee	Dept. of Chemistry, Georgia State University
Prasanth Sambaraju, M.S.	Dept. of Chemistry, UGA
†Dr. Guanqun Yuan	Dept. of Chemistry, UGA
†Dr. Honglei Wang	Dept. of Chemistry, UGA
Dr. Tiandi Zhuang	Dept. of Chemistry, UGA
Dr. Fei Yu	Dept. of Chemistry, UGA
Dr. Shan Liu	Dept. of Chemistry, UGA
Dr. Yihui Zhu	Dept. of Biological and Agricultural Engineering, UGA
Dr. Joshua Rivner	Dept. of Food Science and Technology, UGA
Dr. Xin Li	Dept. of Chemistry, UGA

LABORATORY PERSONNEL

Senior Scientists:

Ramona J. Bieber Urbauer (Research Professional IV), 1999-present

Research Faculty:

Dr. Mario F. Simeonov, Research Associate Professor, 2000-2003, currently Research Associate Professor, University of Kansas

Postdoctoral Researchers:

Dr. Hu Tao (Bill), 2008-present

Dr. Carrie Jolly, 2007-present

Dr. Aaron Cowley, 2004-2006

Awards: - NIH National Research Service Award (postdoctoral fellowship), currently Senior Scientist, OxThera

Dr. Asokan Anbanandam, 2000-2003, currently Postdoctoral Associate, University of Missouri, Dept. of Biochemistry

Awards: - American Heart Association Postdoctoral Fellow, 2001-2003

Graduate Students:

Ryan Bartlett (PhD candidate) 2000-2004, currently Senior Scientist, Monsanto

Awards: - E. L. and Mildred Pursell Wolf Scholarship (2002, KU)

- William King Candlin Memorial Fellowship Award for best senior graduate students in the Department of Molecular Biosciences (2003, KU)

Joshua Gilmore (PhD candidate) 2002-present, currently Postdoctoral Fellow, Stowers Institute for Medical Research

Awards: - Stanley L. Twomey Award for promising graduate students in early/middle stages of graduate career in Department of Molecular Biosciences, (2003, KU)
- Oral Qualifying Exam Honors

Roma Kenjale (PhD candidate) 2001-2003

Awards: - Barbara Johnson Bishop Graduate Scholar (2002, KU)

Jamie Zerbe (PhD candidate) 2002-2003

Awards: - Goldwater Scholarship Awardee (2001)
- Pauline Kimball Prize for Outstanding Woman Senior in Biology (2002, KU)
- Cora Downs Award for Outstanding Female Student, Graduate or Undergraduate (2003, KU)

Jihye Shim (PhD candidate) 2006

Sami Tuomivaara (PhD candidate) 2004-2005

Fullbright Students:

Simon Pflug (Universität Stuttgart, Germany) 2002-2003

Research Assistants:

Heather Smallwood, 2000-2001, currently postdoctoral researcher, Washington State University

Joshua M. Gilmore, 2000-2002, currently postdoctoral researcher, Stowers Institute

Milagros Medina-Duarte Strickland, 2007

Nathan Olive, 2007-2008, currently graduate student

Jane Ullah, 2007-2008

Madeline Elliott, 2008-present, beginning medical school, fall 2009

Exchange students:

Sami Tuomivaara (University of Oulu, Finland) 2000-2001, currently graduate student, University of Georgia

Salaried Undergraduate researchers:

Sara Rosasco, 2001-2002, recently earned PhD, University of Virginia

Jessica Hattle (BS Biochemistry) 2002-2003, currently graduate Student, University of Colorado-Boulder

Awards: - Paul A. Kitos Award for Excellence in Undergraduate Research and Academics (2002, KU)

Marie Cross, 2007-2008, beginning dental school, fall 2009

Awards: - UGA Charter Scholar
- UGA Honors Program

Benjamin Crane, 2006-present

Awards: - UGA Honors Program
- CURO Scholar
- HOPE Scholar

John Galdo, 2006-2007, second year pharmacy student

Awards: - HOPE Scholar

Madeline Elliott, 2006-2007, beginning medical school, fall 2009

Awards: - UGA Honors Program
- CURO Scholar
- HOPE Scholar

REU Students:

Shawgi Silver (Whitman University, Walla Walla Washington), Summer, 2004
Torey Scott Harden (Morehouse University, Atlanta, Georgia), Summer, 2005

SURO (Summer Undergraduate Research Opportunities, Chemistry Dept., UGA) Students:

Savannah Johnson (Piedmont College), Summer, 2007
Noelle Cheung (Carnegie Mellon University), Summer, 2008
Leah Cho (Denver University), Summer, 2008

Undergraduate Researchers/Independent Study:

Aisha Mahmood, 2008-present

Awards: - UGA Honors Program
- CURO Scholar
- HOPE Scholar

Jackie Lastra, 2008-present

Awards: - UGA Honors Program
- CURO Scholar
- HOPE Scholar
- Hispanic Scholarship Fund

Michael Nooromid, 2007-present

Awards: - UGA Honors Program
-HOPE Scholar

Marie Cross, 2007

Awards: - UGA Charter Scholar
- UGA Honors Program

Benjamin Crane, 2006-present

Awards: - UGA Honors Program
- CURO Scholar
- HOPE Scholar

John Galdo, 2006-2007

Awards: - HOPE Scholar

Madeline Elliott, 2006-2007

Awards: - UGA Honors Program
- CURO Scholar
- HOPE Scholar

Neil Patel, 2006

Kimberly Indovina (seeking BS in Biochemistry) 2002-2003

Awards: - National Merit Scholar
- University Scholar (one of 20 outstanding sophomore students selected as University Scholars, 2002-2004, KU)

Timothy Donohue, 2001, currently research technician, University of Colorado Health Sciences Center, accepted to MD/PhD program

Chad McClintick, 2001-2002, currently applying to MD programs

Josh Klemp, 2000, currently medical school student, University of Kansas Medical School

Kay Minn, 2000

Other

Michael Christopher Yonz, 2006
-directed Mr. Yonz' honors option thesis for BCMB3100

COLLABORATORS

Current

Prof. William Lanzilotta, University of Georgia
Prof. Lance Wells, University of Georgia
Prof. Louise Wicker, University of Georgia
Prof. Suren Tatulian, University of Central Florida
Prof. Konstantin Severinov, Waksman Institute, Rutgers University
Prof. Michal Zolkiewski, Kansas State University
Dr. Vladimir Akoev, Kansas State University

Previous

Prof. Ann Hochschild, Harvard University
Prof. Edward N. Brody, SomaLogic (Boulder, CO)
Dr. Karen Adelman, Cornell University
Prof. Michal Zolkiewski, Kansas State University
Dr. Vladimir Akoev, Kansas State University
Prof. Tomasz Heyduk, Saint Louis University
Prof. Teruna Siahaan, University of Kansas
Prof. Ernst Schönbrunn, University of Kansas
Prof. Audrey Lamb, University of Kansas
Prof. Jenny Yang, Georgia State University
Prof. Thomas C. Squier, Pacific Northwest Labs
Prof. Carey K. Johnson, University of Kansas

SEMINARS BY INVITATION

November 14, 2007	Invited seminar – declined due to teaching conflicts Society for Free Radical Biology and Medicine, Washington, DC.
Fall, 2007	Invited seminar – declined due to teaching conflicts Medical College of Wisconsin, Milwaukee, WI.
April 4, 2006	*"New mechanistic insights into prokaryotic transcription regulation by the Anti-Sigma Factor AsiA"*. Department of Molecular and Cellular Biochemistry, The Ohio State University.
November 11, 2005	"*Conformational and Functional Switching of Calmodulin by Methionine Oxidation*" Southeast Magnetic Resonance Conference (SEMRC) 2005, November 10-12, Atlanta, GA.
April 23, 2005	"*Mediating Calcium Signaling by Methionine Oxidation: Nonproductive Interaction of Calmodulin with the Plasma Membrane Ca^{2+}-ATPase Following Oxidation of Methionine Residues in Calmodulin*" First Annual Atlanta Calcium Signaling Symposium, Atlanta, GA.
April 11, 2005	"*Regulating prokaryotic transcription by the novel anti-sigma factor AsiA: structure and function of AsiA and AsiA-polymerase*

	interactions" Department of Biological Sciences, Louisiana State University, Baton Rouge, LA.
November 19, 2004	"*Prokaryotic transcription regulation by the novel anti-sigma factor AsiA*", Department of Chemistry, Georgia State University, Atlanta, GA.
May 6, 2004	"*Transcription regulation by the novel anti-sigma factor AsiA: structure, stability and function of AsiA and AsiA-polymerase interactions*", Department of Biochemistry, University of Iowa, Ames, Iowa.
February 24, 2004	"*Structure, Stability, and Function of the Anti-σ Factor AsiA and the AsiA-σ^{70} Complex*" Department of Biophysics, University of Central Florida, Orlando, Florida.
July 14, 2003	"*Practical aspects of protein structure determination using NMR*" From Cloning to Crystallization: A COBRE workshop, July 14-15, University of Kansas, Lawrence, Kansas.
June 26, 2003	"*Regulation of Transcription by the Anti-sigma factor AsiA*" FASEB Summer Research Conference: *Prokaryotic Transcription Initiation*, June 21-26, Saxton's River, Vermont.
May 28, 2003	"*Regulation of target protein action through oxidative modification*", Workshop on Proteomic Approaches to Oxidative Stress and Biological Aging, Pacific Northwest National Laboratories, Richland, Washington.
Februrary 27, 2003	"*Mechanism of prokaryotic transcription regulation by the anti-sigma factor AsiA*", Pacific Northwest National Laboratories, Richland, Washington
Februrary 24, 2003	"*Mechanistic Insights into Prokaryotic Transcription Regulation and the Role of the Anti-Sigma Factor AsiA*", Department of Chemistry, Rensselaer Polytechnic University, Troy, New York
February 19, 2003	"*New Insights into Prokaryotic Transcription Regulation and the Function of the Anti-Sigma Factor AsiA*". Department of Chemistry and Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia
August 19, 2002	"*Structure and Function of the Anti-sigma Factor AsiA*". 16th Annual Symposium of the Protein Society, San Diego, California
May 10, 2002	"*Mediating Transcription by Subunit Exchange: The Interaction of the Anti-Sigma Factor AsiA with Sigma-70*", Department of Biochemistry, University of Missouri-Columbia, Columbia, Missouri
April 26, 2002	"*Solution Structure and Function of the Anti-Sigma Factor AsiA*". Department of Biochemistry and Molecular Biology, University of Kansas Medical Center, Kansas City Kansas
November 30, 2001	"*Sigmas, Anti-Sigmas, and Antibiotics: Structural Biology of Transcription Regulation by the Anti-Sigma Factor AsiA*". Higuchi Biosciences Center Fall Science Talks, Lawrence, Kansas
July 15, 2001	"*Structure and Stability of the Anti-Sigma Factor AsiA*". FASEB Summer Research Conference: *Prokaryotic Transcription Initiation*, July 14-19, Saxton's River, Vermont.
March 27, 2000	"*Calmodulin and its Interactions with Calmodulin Binding Proteins: NMR studies of Structure, Energetics and Dynamics*". Department of Biochemistry, Kansas State University, Manhattan, Kansas.
February 23, 1999	"*Interactions of Calmodulin with Calmodulin-Binding Domains: Structure, Dynamics, and Energetics*". Department of Molecular Biosciences, University of Kansas, Lawrence, Kansas.
February 8, 1999	"*Molecular Recognition by Calmodulin: Structure, Energetics, and Dynamics of Calmodulin-Peptide Interactions*". School of Biological Sciences, University of Missouri-Kansas City, Kansas City, Missouri.
March 26, 1998	"*Structure and Dynamics of AsiA and the AsiA-σ^{70} Complex*"

	Young Investigator Research Seminars, Department of Biochemistry and Biophysics and Johnson Research Foundation, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania.
February 24, 1996	"*Recent Studies of Calmodulin and Calmodulin Complexes with Calmodulin Binding Domains*" Interdisciplinary Center for Biotechnology Research, University of Florida, Gainesville, Florida

OTHER SEMINARS

September 4, 2001	"*Structure and Function of the Anti-sigma Factor AsiA*". Department of Molecular Biosciences, Biochemistry and Biophysics Section Faculty Seminar Series, University of Kansas, Lawrence, Kansas.
November 13, 2000	"*NMR Studies of the Interaction of an Anti-Sigma Factor with its Target*". Department of Chemistry, Analytical Chemistry Seminar Series, University of Kansas, Lawrence, Kansas.
November 7, 2000	"*Transcription Regulation by AsiA: Solution Structure of AsiA and Elucidation of the AsiA-σ^{70} Interface*". Department of Molecular Biosciences, Biochemistry and Biophysics Section Faculty Seminar Series, University of Kansas, Lawrence, Kansas.
June 4, 1999	"*Calmodulin: Old Dog, New Tricks*". Department of Biochemistry and Biophysics Friday Research Discussions Seminar Series, University of Pennsylvania School of Medicine

PUBLICATIONS

Reviews/Book Chapters

Wand, A. J., Urbauer, J. L., Ehrhardt, M. R., and Lee, A. L. (1999) NMR studies of protein-peptide complexes: Examples from the calmodulin system. *Peptide and Protein Drug Analysis* in *Drugs and the Pharmaceutical Sciences* **101** (November 12), Ch. 23, pp. 727-752, Marcel Dekker, Inc., Ronald E. Reid editor.

Wand, A. J., Urbauer, J. L., McEvoy, R. P., and Bieber, R. J. (1997) Internal Dynamics of Human Ubiquitin Revealed by ^{13}C-Relaxation Studies of Randomly Fractionally Labeled Protein. *Techniques in Protein Chemistry* **VIII**, Academic Press, Daniel R. Marshak, editor, pp. 715-725.

Leopold, M. F., Urbauer, J. L., and Wand, A. J. (1994) Resonance Assignment Strategies for the Analysis of NMR Spectra of Proteins. *Molecular Biotechnology* **2**, 61-93.

Research Publications

Gilmore, J. M., Bieber Urbauer, R. J., Akoev, V., Minakhin, L., Zolkiewski, M., Severinov, K., and Urbauer, J. L. (2009) Determinants of stability and reactivity of the T4 Bacteriophage AsiA Protein. *Biochemistry* (**ready for submission**).

Tatulian, S. A., Bieber Urbauer, R. J., Smallwood, H. S., Bartlett, R. K., Urbauer, J. L., and Squier, T. C. (2009) Oxidation of Met^{144} and Met^{145} in calmodulin blocks alpha-helix formation within the calmodulin-binding sequence of the plasma membrane Ca-ATPase normally associated with calmodulin binding and enzyme activation. *Biochemistry* (**ready for submission**).

Lee, H., Rivner, J., Urbauer, J. L., Garti, N., and Wicker, L. (2008) De-esterification pattern of Valencia orange pectinmethylesterases and characterization of modified pectins. *J. Sci. Food Agric.* **88**, 2102-2110.

Slaughter, B. D., Bieber Urbauer, R. J., Urbauer, J. L., and Johnson, C. K. (2007) Mechanism of Calmodulin Recognition of the Binding Domain of Isoform 1b of the Plasma Membrane Ca^{2+}-ATPase: Kinetic Pathway and Effects of Methionine Oxidation. *Biochemistry* **46**, 4045-4054.

Cowley, A. B., Bieber Urbauer, R. J., and Urbauer, J. L. (2005) ^{1}H, ^{13}C and ^{15}N NMR assignments for AlgH, a putative transcriptional regulator from *Pseudomonas aeruginosa*. *J. Biomol. NMR* **33**, 74-74.

Anbanandam, A., Bieber Urbauer, R. J., Bartlett, R. J., Smallwood, H. S., Squier, T. C., and Urbauer, J. L. (2005) Mediating molecular recognition by methionine oxidation: Conformational switching by oxidation of methionine in the carboxyl-terminal domain of calmodulin. *Biochemistry* **44**, 9486-9496.

Bieber Urbauer, R. J., Rosasco, S. E., Gilmore, J. M., Hattle, J. M., Cowley, A. B. and Urbauer, J. L. (2005) Cloning and high-yield overexpression and purification of AlgH, a regulator of alginate biosynthesis in *Pseudomonas aeruginosa*. *Prot. Expr. Purif.* **43**, 57-64.

Yang, W., Wilkins, A. L., Ye, Y., Liu, Z.-R., Urbauer, J. L., Hellinga, H. W., Kearney, A. van der Merwe, P. A., and Yang, J. J. (2005) Design of a calcium-binding protein with desired structure and cell adhesion function. *J. Am. Chem. Soc.* **127**, 2085-2093.

Osborn, K., Bartlett, R. K., Bieber Urbauer, R. J., Urbauer, J. L., and Johnson, C. K. (2004) Single-molecule dynamics reveal an altered conformation for the autoinhibitor domain of plasma-membrane Ca^{2+}-ATPase bound to oxidatively modified calmodulin. *Biochemistry* **43**, 12937-12944.

Gregory, B., Nickels, B. E., Garrity, S., Severinova, E., Minakhin, L., Bieber Urbauer, R. J., Urbauer, J. L., Heyduk, T., Severinov, K., and Hochschild, A. (2004) A regulator that inhibits transcription by targeting an intersubunit interaction of the RNA polymerase holoenzyme. *PNAS* **101**, 4554-4559.

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